APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 2005, p. 1996–2000 Vol. 71, No. 4
0099-2240/05/$08.00+0 doi:10.1128/AEM.71.4.1996–2000.2005

Effects of Artificial Defoliation of Pines on the Structure and Physiology of the Soil Fungal Community of a Mixed Pine-Spruce Forest

Ken Cullings,[1]* Christopher Raleigh,[1] Michael H. New,[2] and Joan Henson[3]

NASA-Ames Research Center, Moffett Field, California,[1] National Aeronautics and Space Administration Headquarters, Washington, D.C.,[2] and Department of Microbiology, Montana State University, Bozeman, Montana[3]

Received 8 April 2004/Accepted 27 October 2004

Loss of photosynthetic area can affect soil microbial communities by altering the availability of fixed carbon. We used denaturing gradient gel electrophoresis (DGGE) and Biolog filamentous-fungus plates to determine the effects of artificial defoliation of pines in a mixed pine-spruce forest on the composition of the fungal community in a forest soil. As measured by DGGE, two fungal species were affected significantly by the defoliation of pines ($P < 0.001$); the frequency of members of the ectomycorrhizal fungus genus *Cenococcum* decreased significantly, while the frequency of organisms of an unidentified soil fungus increased. The decrease in the amount of *Cenococcum* organisms may have occurred because of the formation of extensive hyphal networks by species of this genus, which require more of the carbon fixed by their host, or because this fungus is dependent upon quantitative differences in spruce root exudates. The defoliation of pines did not affect the overall composition of the soil fungal community or fungal-species richness (number of species per core). Biolog filamentous-fungus plate assays indicated a significant increase ($P < 0.001$) in the number of carbon substrates utilized by the soil fungi and the rate at which these substrates were used, which could indicate an increase in fungal-species richness. Thus, either small changes in the soil fungal community give rise to significant increases in physiological capabilities or PCR bias limits the reliability of the DGGE results. These data indicate that combined genetic and physiological assessments of the soil fungal community are needed to accurately assess the effect of disturbance on indigenous microbial systems.

The rhizosphere supports a wide array of organisms, including bacteria, fungi, and a host of invertebrates. Many of these organisms rely in large part on carbon from root exudates for growth (21, 29, 31, 37). Thus, any above-ground disturbance that alters the availability of fixed carbon (e.g., elevated CO_2, defoliation, and herbivory) could affect the structure and/or function of these soil communities (3, 18, 19, 35, 37, 39, 40, 41, 45, 46, 48).

In this study, we focused on the effects of artificial defoliation on the soil fungal community. The defoliation of plants can either increase carbon allocation to roots, thereby providing increased resources for microbial growth (25, 29), or decrease carbon allocation to roots (22, 42). This relationship depends on whether the plant is grazing tolerant (defoliation results in increased carbon to shoots) or grazing intolerant (defoliation results in increased carbon to roots) (22) and on the age of the plant (12). These relationships can be complicated, however, by root death in response to defoliation, which results in the release of nutrients that can increase the carbon and nitrogen resources available to soil communities and can alter soil pH through the release of organic acids and amino acids (37).

Fungi comprise one of the most important functional groups of soil microbes and are critical to nutrient cycling in terrestrial ecosystems (28, 53). Fungi vary in their carbon demands, and the loss of photosynthetic potential through defoliation can decrease their ability to support and maintain fungal hyphal networks (2). Most studies of defoliation effects on fungal communities have focused on grasses, and the effects can be either positive, e.g., increased root colonization by arbuscular mycorrhizae (12), or negative, e.g., reduced ability to maintain hyphal networks (2). These differences in effects depend on the severity of defoliation, the soil fertility, and the life history strategy of both the fungal species (2, 12, 37) and the defoliated plant.

Few studies have addressed defoliation or carbon sink effects in the context of conifers, although soil fungal biomass is proportionate to photosynthesizing biomass (36). Defoliation decreases fruiting body formation in *Betula* stands (33), decreases ectomycorrhizal (EM) infection of Douglas fir (32) and Pinyon pine (10, 17), and can alter the EM morphotype diversity of Scots pine (49). Artificial defoliation of only pines in the mixed pine-spruce system studied here can significantly alter EM fungal-community structure (9) and significantly affect the EM mututalisms of neighboring, undefoliated spruce (9). Thus, the defoliation of only one tree species in a mixed-species setting can have relatively far-reaching effects (9).

Even if the defoliation effects on fungi are limited to EM interactions, EM fungi often form extensive hyphal networks in the soil that function as nutrient-gathering instruments and effectively increase the surface area across which trees can absorb nutrients (27, 47). In this study, we used a combination of molecular genetic and culture-based methods to determine the effects of defoliation of only pines in a mixed pine-spruce

* Corresponding author. Mailing address: NASA-Ames Research Center, Mountain View, CA 94035-1000. Phone: (650) 604-2773. Fax: (650) 604-3954. E-mail: kcullings@mail.arc.nasa.gov.

forest in Yellowstone National Park on both genetic diversity and functional diversity in the soil fungal community.

We tested the null hypothesis that despite the presence of a second undefoliated tree species supplying root exudates, the defoliation of pines in mixed pine-spruce forest would not significantly affect the soil fungal community. Our rationale was that the presence of undefoliated spruce would buffer the community against the disturbance and that the experiment would provide insight into the role of system complexity in maintaining diversity in the face of disturbance. We used denaturing gradient gel electrophoresis (DGGE) to evaluate changes in soil fungal communities in response to defoliation in terms of the presence or absence and richness of soil fungal species that could be amplified using PCR. We also used filamentous-fungus plates (Fungilog; Biolog, Hayward, Calif.) (11, 16, 23) to determine effects of defoliation on soil fungal physiological biodiversity. These methods provide different measures of fungal diversity, a measure of the fungal DNA resident in the soil, and a measure of the culturable members of the fungal community. DGGE measures diversity of the fungal DNA resident in the soil, while Fungilog growth assays measure the potential physiological capabilities of the fungi that are culturable. This study is the first to combine these methods to study effects of a conifer system on the soil fungal community and to take the possible moderating effects due to aboveground species diversity into account.

MATERIALS AND METHODS

In the summer of 1998, individuals of *Pinus contorta* (lodgepole pine) in a mixed *P. contorta-Picea engelmannii* (Engelmann spruce) stand in Yellowstone National Park, Wyo., were 50% defoliated by removing every other pair of needles at the base of each whorl. The study site was in a forest approximately 100 m from the shores of Yellowstone Lake, and the soils were composed of obsidian sand. Coring sites were along the margin of a clear-cut area in which the litter layer and understory were identical to those of undisturbed forest (a 3- to 5-cm litter layer and understory of *Vaccinium scoparium* Cov [grouse whortleberry]) that has been the subject of previous studies (8, 9). The clear-cut was created by the National Park Service approximately 50 years ago, when logs were required to build structures in the park (Bob Lindstrom, Yellowstone Center for Resources, personal communication). Treatment was performed in three paired treatment-control blocks.

Each paired treatment and control block was 5 by 3 m, and each pair was separated from the surrounding forest by at least 5 m to eliminate edge effect. Treatment and control blocks were situated directly adjacent to each other along a 30-m transect, and the three replicate paired blocks were all within 5 m of each other. This spatial arrangement was arrived at empirically (8, 9), was designed to decrease the likelihood that spatial variation in the fungal community would overshadow treatment effects, and has been used by us in the past to assess effects of artificial defoliation on the EM community of this stand (9).

Trees of both *P. contorta* and *Picea engelmannii* species had reseeded naturally and were 1 to 2 m in height, while *P. contorta* stems significantly outnumbered *Picea engelmannii* stems ($P < 0.001$). Control blocks contained 60 ± 17 (standard error) *P. contorta* individuals and 7 ± 2 *Picea engelmannii* individuals, while treatment blocks contained 68 ± 16 and 10 ± 2 individuals, respectively. All individuals of *P. contorta* in treatment plots were 50% defoliated by twisting at the base of every other needle whorl over the entire canopy.

For DGGE analysis, seven 15-ml soil samples (approximately 2 cm in diameter and 12 cm deep; number of cores, 21) were taken from within each block, preserved on dry ice for 3 days (until they were returned to the lab), and then freeze-dried for long-term storage. Roots were concentrated in the top few centimeters of each core, and soils for analysis were taken from the root zone. DNA was extracted from 10-mg soil samples (containing no root material) by using sonication and a detergent-based mini-prep (7). Detergent-based preps of small amounts of soil can provide resolution down to 1 culturable cell per 10 mg of soil by PCR-based assays (6). To detect fungal DNA in the soil extract, the universal fungal primer set ITS1F-ITS4 was used (13). All reactions were successful. The parameters for PCR were as follows: initial denaturation at 95°C for 1 min 35 s; 13 cycles of denaturation at 94°C, primer annealing for 55 s at 55°C, and polymerization for 45 s at 72°C; 9 additional cycles with the polymerization time extended to 2 min; 9 cycles with a 3-min extension; and a final 10-min polymerization step at 72°C.

Amplified fungal DNA from soils were subjected to DGGE to separate fungal taxa and make identifications of individual fungal species possible. PCR amplification products were gel purified on agarose gels (2% low-melting temperature gels and 1% unmodified gels), the bands were excised with a sterile razor blade, the gel fragments were transferred to individual 1.5-ml Eppendorf tubes with 200 μl of Tris-EDTA buffer, and the tubes were heated to 65°C for 10 min to melt the agarose gel matrix and free the DNA. To ensure consistency within samples, the analysis of sample extracts ($n = 14$) from the first set of paired blocks was repeated three times with each sample. There was no variation among samples; thus, each of the remaining samples was assayed once.

For each DGGE assay, 12.5 μl of a 1:10 dilution of amplified DNA in Tris-EDTA (10 ml of 1 M Tris [pH 8.0] plus 4 ml of 0.2 5 M EDTA [pH 8.0] in 986 ml of distilled H_2O) was used as a PCR template in a reaction to add GC clamp tails. The PCR protocol was the same as before except that one primer (ITS1F) had an additional 40-bp GC tail.

A DCode DGGE universal detection system apparatus (Bio-Rad, Hercules, Calif.) was used to perform the DGGE analysis according to the manufacturer's specifications and as previously described (13), except that a 20 to 60% denaturant gradient was used. Individual bands were cut out and sequenced on an automated sequencer (ABI Prism 377; Perkin-Elmer, Boston, Mass.) at California State University, Hayward. Sequence data were run through the BLAST search programs of the National Center for Biotechnology Information (www4.ncbi.nlm.nih.gov/) to determine the taxonomic affiliation of each DGGE band by sequence homology.

To determine the functional diversity of the soil fungal community, we used Fungilog microtiter plates. For each assay, a 10-g soil sample (three from each of the treatment and control blocks; a total of nine samples for each experiment) was shaken in 100 ml of Ringer's solution (20) (three times for 1 min each). This solution was allowed to settle for 15 min, and 150-μl aliquots were pipetted onto the Fungilog microtiter plates. Each plate contained 95 wells with different carbon sources and a single control well with no carbon source. Plates were incubated at room temperature with antibiotics (50 μg each of streptomycin and ampicillin/ml) to ensure that results were due to fungal and not bacterial growth. Every 24 h, for 5 days, plates were scanned with an optical density plate reader (at 490 nm). This method produces 96 data points (95 substrates plus one water control) per replicate, per day, with each corresponding to a specific carbon substrate. Growth is measured colorimetrically when the tetrazolium dye in the medium accepts electrons from active electron transport. Plates were read before growth became too massive, that is, before overrunning the plates and becoming nonlinear.

The significance of the effects of defoliation on the presence or absence and abundance of soil fungal species was determined with a contingency table and χ^2 analysis for the whole community and Fisher's exact test for the species that exhibited differences following treatment. Changes in species richness, defined as the number of unique DGGE bands (or fungal species) per unit area (in this case per soil sample), were analyzed by using Student's t test. Principal-components analysis (PCA) was used to project the data onto a small number of statistically independent axes that account for the majority of the variability in the data. This projection results from identification of the eigenvectors of the covariance matrix of the data. Then the data were projected onto the eigendirections with the largest eigenvalues. The significance of the effects on physiological diversity, as indicated by total number of substrates utilized and rates of substrate utilization, was determined with Student's t test, with each day being analyzed individually.

RESULTS

The defoliation of only pines in a mixed pine-spruce forest resulted in no significant effect on species richness of the predominant soil fungal species (number of species per sample). Both control samples and treatment samples contained five species per sample (±0.4 for controls, and ±0.5 for treatments) as indicated by DGGE analysis. Likewise, χ^2 analysis of the total community indicated no significant effect on overall soil fungal-community composition; the majority of fungal species were present at the same frequency in both treatment

TABLE 1. Presence or absence and abundances of fungal taxa

DGGE band	GenBank accession no.	% Similarity	Taxon[c]	No. of cores (n = 21)[a]	
				Treatment	Control
1	AY574924	90–100	*Tylospora* sp.	12	12
2	AY576782	90–100	*Tylospora* sp.	13	13
3			Not sequenced[d]	0	1
5	AY574921	90–100	*Piloderma* sp.	6	6
6			Not sequenced[d]	1	0
7	AY576780	90–100	*Piloderma* sp.	8	10
8[b]	AY574922		No close affiliation	9	3
9[b]	AY574923	90–100	*Cenococcum* sp.	2	8
10	AY576781	90–100	*Eupenicillium* sp.	8	10
11			Not sequenced[d]	0	1

[a] Abundance is expressed as the number of cores in which each taxon was detected.
[b] P was <0.001 as indicated by a contingency table and χ^2 analysis.
[c] Taxons are as indicated by a BLAST search.
[d] Taxon was not sequenced due to the low frequency of observance.

blocks and control blocks (Table 1). Most BLAST analyses indicated that the DGGE bands were ≥95% similar (excluding unknown bases) to those of EM fungi of conifers. The two exceptions were taxon 10, which had high sequence similarity (≥95%) with a species of the genus *Eupenicillium*, a free-living ascomycete, and taxon 8, which, though clearly a basidiomycetous fungus, was not closely related to any fungus represented by a sequence in GenBank (82% match to any fungus in GenBank and to only 300 bases of more than 600 sequenced). This sample was sequenced from multiple amplifications and in both directions in order to rule out the presence of chimeras.

Although species richness was not affected, there was a significant effect ($P < 0.001$) when the frequency of individual fungi was analyzed with Fisher's exact test. Specifically, *Cenococcum* sp. (taxon 9, an ascomycete fungus that is an important EM associate of pines) was common in control plots but nearly eliminated following treatment, while the unidentified taxon 8 was relatively rare in controls but common in treatment blocks.

The defoliation of only pines in a mixed pine-spruce forest resulted in significant changes in physiological capacity as measured by Fungilog tests ($P < 0.001$), both in terms of carbon source utilization (with growth being indicated by tetrazolium dye) (Fig. 1A) and in the number of carbon substrates utilized (Fig. 1B). Both measures were significantly higher in treatment samples from 72 h until the end of the experiment. Repeated measures with a further sampling of equal size produced similar, statistically significant results.

PCA placed samples into one cluster (Fig. 2) that contained 8 of 9 control cores and three treatment cores. The remaining cores were in a relatively loose assemblage that did not form a cohesive cluster and were distinct and separate from the control cluster. Separation of the control cluster was based primarily on greater utilization of sugars. The first two principal components accounted for 72 and 10% of the variation in the data. The first component was correlated with 22 sugars (*n*-acetyl-D-glucosamine, D-arabitol, dextrin, D-fructose, galactose, D-galacturonic acid, gentiobiose, D-gluconic acid, D-glucose, 2-keto-gluconic acid, maltose, D-mannitol, D-mannose, palatinose, D-melezitose, sucrose, D-trehalose, D-xylose, D-cellobiose, glycogen, D-glucuronic acid, and D-ribose), seven fatty acids (Tween 80, ketoglutaric acid, L-lactic acid, L-maleic acid,

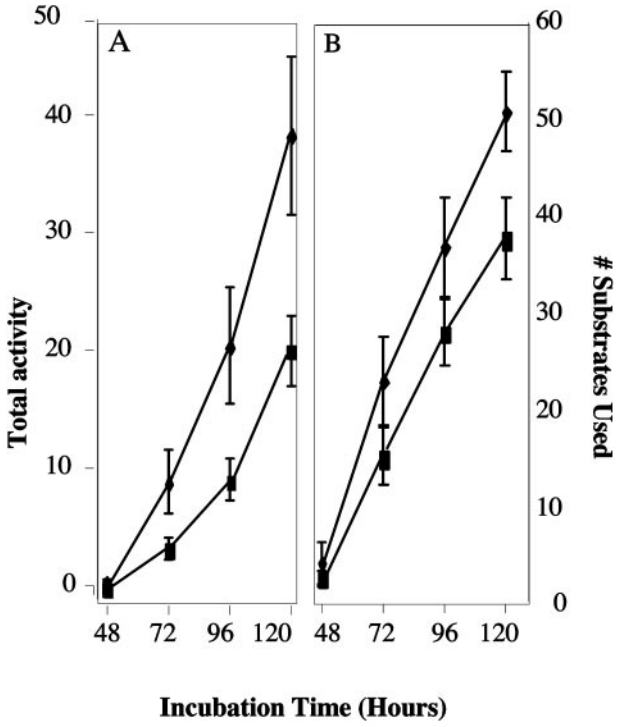

FIG. 1. Results of Biolog filamentous-fungus assays. (A) Total activity; (B) number of substrates utilized. ◆, treatments; ■, controls. Only results from days 2 to 5 are depicted, as activities on day 1 were zero.

quinic acid, D-saccharic acid, and succinic acid), and seven amino acids (L-alanine, L-alanylglycine, L-asparagine, L-phenylalanine, L-glutamic acid, glycyl-L-glutamic acid, and L-serine), while the second component was correlated with 15 sugars (D-arabitol, dextrin, D-fructose, gentiobiose, D-gluconic acid, 2-ketogluconic acid, maltose, D-mannitol, D-mannose, D-melezitose, palatinose, sucrose, D-trehalose, and D-ribose), seven fatty acids (Tween 80, ketoglutaric acid, L-lactic acid, L-maleic acid, quinic acid, D-saccharic acid, and succinic acid), and five amino acids (L-alanine, L-glutamic acid, glycyl-L-glutamic acid, L-serine, and uridine [a nucleic acid]).

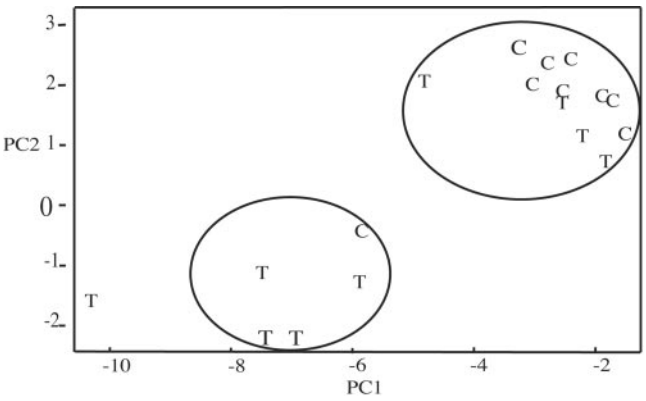

FIG. 2. PCA of Fungilog data. T corresponds to treatments, and C corresponds to controls.

DISCUSSION

Changes in below-ground allocation of carbon resulting from above-ground disturbances (such as defoliation and herbivory) can alter both the structure and the physiological diversity of microbial communities (e.g., references 3, 12, 37, 39, 40, 41, 45, and 46).

DGGE analysis indicated that defoliation of only pines in experimental blocks of a mixed stand of pine and spruce can affect the abundance of individual soil fungal species but that species richness (number of species per core) and overall soil fungal-community composition are not significantly affected. Most of the fungi detected by DGGE have high sequence similarity to EM fungi, with a few being similar to free-living fungal taxa. None of the fungi with high sequence similarity to free-living fungi were affected by defoliation.

One fungus that was affected by defoliation, *Cenococcum*, was more abundant in controls, and a second, taxon 8, was more abundant in treatments and thus appeared to respond positively to the changes induced by defoliation. *Cenococcum* is a well-studied EM fungus that is very common in pine forests, although little is known about how this fungus reacts to defoliation. Since *Cenococcum* can form a large biomass and extensive rhizomorphs in forest soil (14, 30), a reduction in photosynthetic potential and concomitant reduction in the fixed carbon allocated to the roots of the EM plants could reduce the amount of this fungus present.

We also hypothesize that the presence of nondefoliated spruce helped maintain fungal-species richness in these nutrient-poor soils and may also have contributed to the measured increase in *Cenococcum* organisms. Our data suggest that roots of the nondefoliated spruce tree can support the EM fungi of the defoliated pines (9) and hence help to maintain EM fungal-community composition and infection levels. In addition, growth of fungal endophytes can be influenced by the presence of specific host plant carbohydrates (24). Thus, *Cenococcum* may respond more readily to spruce root exudates and increase in frequency when roots of spruce proliferate following pine defoliation (9).

The Fungilog experiments indicated significant changes in the physiological capabilities of the fungal communities. PCA clustered 8 of 9 control cores into a distinct group, while most of the treatment cores were in a looser assemblage that was distinct from the control group. These groupings were based primarily on differences in carbohydrate utilization. The single control core that was separate from the main control group was taken near the edge of adjacent treatment and control blocks, and the hyphae could have crossed over into adjoining blocks. Three of the nine treatment cores also clustered with the majority of the control cores. We hypothesize that these cores contained hyphae responding to exudates from spruce, or communities that were still in transition, despite the passage of 2 years.

Fungilog results also indicated increased physiological diversity in response to defoliation in the form of increases in both the range of utilizable substrates and their rate of utilization. This change occurred despite the presence of the second undefoliated tree species. Thus, quantitative and qualitative differences in root exudates of the two tree species may play a role in influencing fungal-community structure and function.

However, Fungilog results differ from those of the DGGE analysis, which indicated very little change in species composition and richness. The discrepancy between the two methods may be due to two factors. First, if a single fungal species has a wide range of carbon substrate usage capabilities, the relatively large changes in physiological diversity may result even with only minor changes in fungal-community composition. Fungi can have very different niche sizes, defined as the number of carbon substrates each species or strain can utilize. Under some conditions, this can range from 18 of 95 Biolog substrates utilized to 92 of 95 Biolog substrates utilized (34). Studies of fungal endophytes of conifers are consistent with these observations and indicate that strains specific to petioles, for example, have a much broader substrate utilization capability than do those restricted to needles (4). Thus, the increase in taxon 8 organisms might suffice to produce the Fungilog patterns that we observed. We will test this hypothesis by assessing the physiological capabilities of the fungus that increased in frequency when or if the fruiting bodies needed for culturing this fungus become available.

A second explanation for the differences between Fungilog results and DGGE results could be PCR amplification bias. This bias occurs when primers are used in multiplex reactions (50), particularly if the fungi present in the soil lack sequences that correspond exactly to the primers used. Our primers have been tested extensively against fungi across the *Eumycota* (15), so we consider this possibility unlikely. However, even when perfect sequence homology exists, other factors may inhibit amplification. For example, the secondary structure of the DNA flanking the priming site can cause DNA from some species to be preferentially amplified (26), and thus some species might not be detected, even if they are present in relatively high numbers. We compared the sequences obtained in this study with those obtained in our previous study of EM in these study plots (9). None of the EM fungal species detected in our previous study were present in our DGGE analysis of the same soils. Thus, the EM fungi that we detected in our previous study form little or no hyphal biomass in soils, or there was extensive turnover in the EM fungal community. We plan to test the primer bias hypothesis by repeating the Fungilog assays and then sequencing and identifying the fungi that are cultured on each substrate.

In summary, our data indicate that the defoliation of only pines in a mixed pine-spruce study site can significantly affect the relative abundance of some soil fungal species, though overall species richness and composition are largely unaffected. Furthermore, the defoliation had a significant effect on the physiological diversity of the soil fungal community. However, these results indicate that care must be taken when interpreting the data and that independent assessments of genetic diversity within Fungilog cultures of the type that we plan to perform may be useful. Given the increasing importance placed on studies of biodiversity and physiological diversity, and roles of biodiversity and physiological diversity in ecosystem resilience (e.g., references 1, 5, 36, 38, 43, 44, 51, and 52), it is clear that studies utilizing methods that measure both species composition and species physiological diversity are needed.

ACKNOWLEDGMENTS

This work was supported by an NSF grant (DEB/RUI 9420141) to Ken W. Cullings and Virgil T. Parker and a NASA-DDF grant to Ken W. Cullings.

We thank J. R. Blair for mushroom identifications, Bob Lindstrom and Ann Deutch of the Yellowstone Center for Resources, Ann Rodman of the Yellowstone Soil Survey, Alan Kerner for logistical support, and Chris Baysdorfer of California State University, Hayward, for DNA sequencing.

REFERENCES

1. **Allen, E. B., M. F. Allen, D. J. Helm, J. M. Trappe, R. Molina, and E. Rincon.** 1995. Patterns and regulation of mycorrhizal plant and fungal diversity. Plant Soil **170:**47–62.
2. **Allsopp, N.** 1998. Effect of defoliation on the arbuscual mycorrhizas of three perennial pasture and rangeland grasses. Plant Soil **202:**117–124.
3. **Behera, N., and R. S. Patnaik.** 1980. Rhizosphere microflora of intact and defoliated plants of *Dolichos biflorus* L. Acta Bot. Indica **8:**105–109.
4. **Carroll, G., and O. Petrini.** 1983. Patterns of substrate utilization by some fungal endophytes from coniferous foliage. Mycologia **75:**53–63.
5. **Collins, S. L., and T. L. Benning.** 1996. Spatial and temporal patterns in functional diversity, p. 253–280. *In* K. J. Gaston (ed.), Biodiversity: a biology of numbers and difference. Blackwell Scientific Publications, Cambridge, Mass.
6. **Cullen, D. W., and P. R. Hirsch.** 1998. Simple and rapid method for direct extraction of microbial DNA from soil for PCR. Soil Biol. Biochem. **30:**983–993.
7. **Cullings, K. W.** 1992. Design and testing of a plant-specific PCR primer for ecological and evolutionary studies. Mol. Ecol. **1:**233–240.
8. **Cullings, K. W., D. R. Vogler, V. T. Parker, and S. K. Finley.** 2000. Ectomycorrhizal specificity patterns in a mixed *Pinus contorta* and *Picea engelmannii* forest in Yellowstone National Park. Appl. Environ. Microbiol. **66:**4988–4991.
9. **Cullings, K. W., D. R. Vogler, V. T. Parker, and S. Makhija.** 2001. Defoliation effects on the ectomycorrhizal community of a mixed *Pinus contorta*/*Picea engelmannii* stand in Yellowstone Park. Oecologia **127:**533–539.
10. **Del Vecchio, T. A., C. A. Gehring, N. S. Cobb, and T. G. Whitham.** 1993. Negative effects of scale insect herbivory on the ectomycorrhizae of juvenile Pinyon pine. Ecology **74:**2297–2302.
11. **Dobranic, J. K., and J. C. Zak.** 1999. A microtiter plate procedure for evaluating fungal functional diversity. Mycologia **91:**756–765.
12. **Eom, A.-H., G. W. T. Wilson, and D. C. Hartnett.** 2001. Effects of ungulate grazers on arbuscular mycorrhizal symbiosis and fungal community structure in tallgrass prairie. Mycologia **93:**233–242.
13. **Ferris, M. J., G. Muyzer, and D. M. Ward.** 1996. Denaturing gradient gel electrophoresis profiles of 16S rRNA-defined populations inhabiting a hot spring microbial mat community. Appl. Environ. Microbiol. **62:**340–346.
14. **Fogel, R., and G. Hunt.** 1979. Fungal and arboreal biomass in a western Oregon USA Douglas-fir (*Pseudotsuga menziesii*) ecosystem distribution patterns and turnover. Can. J. For. Res. **9:**245–256.
15. **Gardes, M., and T. D. Bruns.** 1993. ITS primers with enhanced specificity for higher fungi and basidiomycetes: application to identification of mycorrhizae and rusts. Mol. Ecol. **2:**113–118.
16. **Garland, J. L., and A. L. Mills.** 1991. Classification and characterization of heterotrophic microbial communities on the basis of community-level sole-carbon-source utilization. Appl. Environ. Microbiol. **57:**2351–2359.
17. **Gehring, C. A., and T. G. Whitham.** 1992. Reduced mycorrhizae on *Juniperus monosperma* with mistletoe: the influence of environmental stress and tree gender on a plant parasite and a plant-fungal mutualism. Oecologia **89:**298–303.
18. **Gehring, C. A., and T. G. Whitham.** 1994. Comparisons of ectomycorrhizae on Pinyon pines (*Pinus edulis*; Pinaceae) across extremes of soil type and herbivory. Am. J. Bot. **81:**1509–1516.
19. **Gehring, C. A., and T. G. Whitham.** 1995. Duration of herbivore removal and environmental stress affect the ectomycorrhizae of Pinyon pines. Ecology **76:**2118–2123.
20. **Grayston, S. J., and C. D. Campbell.** 1996. Functional biodiversity of microbial communities in the rhizospheres of hybrid larch (*Larix eurolepis*) and Sitka spruce (*Picea sitchensis*). Tree Physiol. **16:**1031–1038.
21. **Grayston, S. J., D. Vaughan, and D. Jones.** 1996. Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. Appl. Soil Ecol. **5:**29–56.
22. **Guitan, R., and R. D. Bardget.** 2000. Plant and soil responses to defoliation in temperate semi-natural grassland. Plant Soil **220:**271–277.
23. **Haack, S. K., H. Garchow, M. J. Klug, and L. J. Forney.** 1995. Analysis of factors affecting the accuracy, reproducibility, and interpretation of microbial community carbon source utilization patterns. Appl. Environ. Microbiol. **61:**1458–1468.
24. **Hadacek, F., and G. F. Kraus.** 2002. Plant root carbohydrates affect growth behavior of endophytic microfungi. FEMS Microbiol. Ecol. **41:**161–170.
25. **Hamilton, E. W., and D. A. Frank.** 2001. Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass. Ecology **82:**2397–2402.
26. **Hansen, M. C., M. Tolker-Nielsen, M. Givskov, and M. Soren.** 1998. Biased 16S rDNA PCR amplification caused by interference from DNA flanking the template region. FEMS Microbiol. Ecol. **26:**141–149.
27. **Harley, J. L., and S. E. Smith.** 1983. Mycorrhizal symbiosis. Academic Press, London, England.
28. **Hibbett, D. S., L.-B. Gilbert, and M. J. Donoghue.** 2000. Evolutionary instability of ectomycorrhizal symbioses in basidiomycetes. Nature **407**:506–508.
29. **Holland, J. N., W. Cheng, and D. A. Crossley.** 1996. Herbivore-induced changes in plant carbon allocation: assessment of below-ground C fluxes using carbon-14. Oecologia **107:**87–94.
30. **Hunt, G. A., and R. Fogel.** 1983. Fungal hyphal dynamics in a western Oregon USA *Pseudotsuga menziesii* stand. Soil Biol. Biochem. **15:**641–650.
31. **Ingham, E. R., and H. B. Massicotte.** 1994. Protozoan communities around conifer roots colonized by ectomycorrhizal fungi. Mycorrhiza **5:**53–61.
32. **Kolb, T. E., K. A. Dodds, and K. M. Clancy.** 1999. Effect of western spruce budworm defoliation on the physiology and growth of potted Douglas-fir seedlings. For. Sci. **45:**280–291.
33. **Last, F. T., J. Pelham, P. A. Mason, and K. Ingelby.** 1979. Influence of leaves on sporophore production by fungi forming sheathing mycorrhizas with *Betula* spp. Nature **280:**168–169.
34. **Lee, H. B. M., and N. Magan.** 1999. Environmental factors and nutritional utilization patterns affect niche overlap indices between *Aspergillus ochraceus* and other spoilage fungi. Lett. Appl. Microbiol. **28:**300–304.
35. **Markkola, A. M.** 1996. Effect of artificial defoliation on biomass allocation in ectomycorrhizal *Pinus sylvestris* seedlings. Can. J. For. Res. **26:**899–904.
36. **Martinez, N. D.** 1996. Defining and measuring functional aspects of biodiversity, p. 114–148. *In* K. J. Gaston (ed.), Biodiversity: a biology of numbers and difference. Blackwell Scientific Publications, Cambridge, Mass.
37. **Mawdsley, J. L., and R. D. Bardgett.** 1997. Continuous defoliation of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) and associated changes in the composition and activity of the microbial population of an upland grassland soil. Biol. Fertil. Soils **24:**52–58.
38. **McCann, K. S.** 2000. The diversity-stability debate. Nature **405:**228–233.
39. **Mikola, J., G. W. Yeates, G. M. Barker, and D. A. Wardle.** 2000. Linking above-ground and below-ground effects in autotrophic microcosms: effects of shading and defoliation on plant and soil properties. Oikos **89:**577–587.
40. **Mikola, J., G. W. Yeates, G. M. Barker, D. A. Wardle, and K. I. Bonner.** 2001. Effects of defoliation on the soil food-web properties in an experimental grassland community. Oikos **92**:333–343.
41. **Mikola, J., G. W. Yeates, G. M. Barker, D. A. Wardle, and K. I. Bonner.** 2001. Responses of soil food-web structure to defoliation of different plant species combinations in an experimental grassland community. Soil Biol. Biochem. **33**:205–214.
42. **Miller, R. F., and J. Q. Rose.** 1992. Growth and carbon allocation of *Agropyron desortorum* following autumn defoliation. Oecologia **89:**482–486.
43. **Miller, S. L.** 1995. Functional diversity in fungi. Can. J. Bot. **73**(Suppl. 1)**:**S50–S57.
44. **Naeem, S., and S. Li.** 1997. Biodiversity enhances ecosystem reliability. Nature **390:**507–509.
45. **Norby, R. J., E. G. O'Neill, W. G. Hood, and R. J. Luxmore.** 1987. Carbon allocation, root exudation and mycorrhizal colonization of *Pinus echinata* seedlings under elevated CO_2 enrichment. Tree Physiol. **3:**203–210.
46. **Priha, O., T. Lehto, and A. Smolander.** 1999. Mycorrhizas and C and N transformations in the rhizospheres of *Pinus sylvestris*, *Picea abies* and *Betula pendula* seedlings. Plant Soil **206:**191–204.
47. **Read, D. J.** 1991. Mycorrhizas in ecosystems. Experientia **47:**376–391.
48. **Rossow, L. J., J. P. Bryant, and K. Kielland.** 1997. Effects of above-ground browsing by mammals on mycorrhizal infection in an early successional taiga ecosystem. Oecologia **110:**94–98.
49. **Saikkonen, K., U. Ahonen-Jannarth, A. M. Markkola, M. Helander, J. Tuomi, M. Roitto, and H. Ranta.** 1999. Defoliation and mycorrhizal symbiosis: a functional balance between carbon sources and below-ground sinks. Ecol. Lett. **2:**19–26.
50. **Takahiro, K.** 2003. Bias and artifacts in multitemplate polymerase chain reactions (PCR). J. Biosci. Bioeng. **96:**317–323.
51. **Tillman, D., J. Knops, D. Wedin, P. Reich, M. Ritchie, and E. Siemann.** 1997. The influence of functional diversity and composition on ecosystem processes. Science **277:**1300–1302.
52. **Zak, J. C., and S. Visser.** 1996. An appraisal of soil fungal biodiversity: the crossroads between taxonomic and functional biodiversity. Biodivers. Conserv. **5:**169–183.
53. **Zhu, W., and J. G. Ehrenfeld.** 1996. The effects of mycorrhizal roots on litter decomposition, soil biota, and nutrients in a spodosolic soil. Plant Soil **179:**109–118.